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PATENT SPECIFICATION

NO DRAWINGS

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COMPLETE SPECIFICATION

A method for manufacturing Beverages containing Living Lactic Acid Bacilli

We, KABUSHIKI KAISHA YAKULT HONSHA, of No. 6, 3-chome, Nihonbashi, Hon-cho, Chou-ku, Tokyo, Japan, A Japanese Company, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

This invention relates to a method for manufacturing a beverage or a composition for making a beverage containing living lactic acid bacilli by autolysing fresh chlorellae or scenedesmus to produce an autolysed product, treating the autolysed product with water to extract the water-soluble components thus forming an aqueous extract solution and mixing the aqueous extract solution with dextrose or sugar and animal milk to produce a nutrient medium, inoculating and cultivating lactic acid bacilli such as Lactobacillus acidophilus on the nutrient medium to produce a cultured broth and then adding sweetening materials and essences to the cultured broth.

Animal milk as used in this invention is to be taken to include closely allied milk products such as skimmed milk.

In conventional methods for manufacturing beverages containing lactic acid bacilli, it is known that the propagation of lactic acid bacilli is accelerated when they are cultivated in either: a nutrient medium containing animal milk and unicellular green algae such as chlorella or scenedesmus; or an extract solution obtained by extraction of the same with an acidic or alkaline solution. Such a conventional method, however, utilizes only the water soluble substances from the cells of the chlorella or scenedesmus algae for accelerating the propagation of lactic acid bacilli. Of the water soluble substances, the main effective component for accelerating the initial growth of lactic acid bacilli are inorganic salts. The amount of the nitrogen contained in the water soluble substances is less than 5% of the nitrogen contained in the chlorella algae and thus it may be seen that a substantial amount of the nitrogen component remains in the algae and the nitrogen component is not fully utilized. The chlorella contains other effective components such as the following in the stated proportions: protein, about 50%; lipid, 10%; carbohydrate, 20%; ash, about 10%; and also vitamins and other substances and also the protein contains essential amino acids. However, these effective components are not extracted with the aqueous extract as chlorellae have strong cell walls and the vitamins are decomposed when the chlorellae are heated or treated with an alkaline or acidic substance. Moreover, the water insoluble substances of the chlorella algae cannot act to accelerate the growth of lactic acid bacilli by themselves. The inventors have investigated a method for manufacturing beverages containing living lactic acid bacilli which are nutritious and have prophylactic properties by fully utilizing the effective components contained in the chlorella algae. As a result, the inventors have cultivated lactic acid bacilli on the water soluble substances, which are prepared by autolysing fresh chlorellae or scenedesmus and extracting the autolysed water soluble substances, which contain more than 90% of the nitrogen content in the fresh chlorella algae, and have found that the water soluble substances do not only accelerate the growth of lactic acid bacilli, but also prolong the life of the bacilli. Also, the inventors have found that such effects are based on the fact that fresh chlorellae die when maintained at certain temperatures and they are autolysed and their cell walls decomposed, and the protein, nucleic acid and carbohydrates are decomposed into compounds of lower molecular weight due to the activity of the enzymes contained in the chlorellae, but the vitamins are not decomposed. The resulting available components can easily be extracted with water to give an aqueous extract. The

[*Price 4s. 6d.*]

test results of the propagation of lactic acid bacilli are shown in the following Table 1.

TABLE 1

Comparison of the propagation of lactic acid bacilli in a nutrient medium containing an extract solution from chlorellae.							
Culture time in hours	0	24	48	72	96	120	144
Nutrient medium							
Extract solution obtained from the product of autolysis.	0.2	1.4	2.2	2.7	3.0	3.3	3.4
Extract solution obtained using 1% HCl	0.2	0.2	0.4	0.6	0.7	0.9	0.9
Extract solution obtained using water at 100°C for 30 minutes	0.2	0.2	0.4	0.5	0.6	0.7	0.8
Extract solution obtained using 1% NaOH	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Skim milk solution (15%)	0.2	0.6	1.2	1.7	2.0	2.2	2.3

Note: (1) The nutrient medium contains 3% of dextrose and has an initial pH of 6.8.

(2) The extract solution is obtained by treating 10 grams of dried chlorellae.

(3) The numerals showing the growth of lactic acid bacilli are measured by the titration method for acidity.

The inventors have succeeded in the heterotrophic growth of chlorellae or scenedesmus on a large scale by using a tank culture with which it is easy to control the propagation of the chlorellae and to keep the physical conditions constant, so that if the chlorellae obtained by this method are used the autolysis can easily be carried out. It has been found that the cell walls of the autolyzed chlorellae are nearly all decomposed, and the available components contained in the autolyzed chlorellae can be more easily extracted if they are mechanically crushed by a grinder or in a mill or a homogenizer.

A method of this invention will now be explained in detail.

Chlorellae are cultured and then are collected at the peak of their growing period and then are washed with water to obtain a paste. The paste is added to toluene and the resultant mixture is mixed and left for 24 hours at 37°C. Alternatively, the paste may be suspended in a pH 7.0 buffer solution and then toluene added with shaking. The toluene may be replaced by chloroform or ethyl acetate. Also a concentrated algae paste may be autolysed without addition of a solvent such as toluene. Thus, the chlorellae are autolyzed and after being ground, they are treated with water, as extractant, to obtain an aqueous extract solution, while non-autolyzed chlorellae and solvent (toluene) are removed or separated by centrifugal separation. The extract solution is a brownish translucent liquid having a sweet flavour and containing amino acids, peptides, nucleotides, proteins, vitamins, carbohydrates such as sugar, and inorganic salts. It has been found that more than 90% of the nitrogen and more than 50% of sugar which are contained in the algae are extracted from the algae by this method. The results achieved by the autolysis of the chlorellae algae in different samples and by different treatments are shown in the following Table 2.

TABLE 2

Extraction ratio of nitrogen by autolysis. (The extraction ratio of the nitrogen is calculated by the equation:

$$\text{Extraction} = \frac{\text{Nitrogen in the extract solution}}{\text{Nitrogen in the algae}} \times 100\%)$$

Samples	Treating conditions	Extraction ratio of nitrogen (%)
Autotrophically grow chlorellae	Water extraction at room temperature for 1 hour	0
" "	Autolyzis with toluene at 37°C for 24 hours	44
" "	Extraction with 1% HCl at 100°C for 5 minutes	0.5
" "	Extracted with 1% HCl at 37°C for 24 hours	3.1
Heterotrophically grow chlorellae	Water extraction at room temperature for 1 hour without agitation	0
" "	Autolyzis with toluene at 37°C for 24 hours without agitation	82
" "	Autolyzis with toluene at 37°C for 24 hours with shaking	62
" "	Autolyzis with ethyl acetate at 37°C for 24 hours without agitation	73
" "	Autolyzis with ethyl acetate at 37°C for 24 hours with shaking	54

It was found that when the nutrient medium containing the aqueous extract obtained by the autolysis treatment, sugar, and animal milk was inoculated with lactic acid bacilli the lactic acid bacilli multiplied considerably and had remarkable longevity.

Further, the accelerating effect on the growth of lactic acid bacilli was measured and also the change in the numbers of living bacilli was measured. The experimental results are shown in Tables 3 and 4.

TABLE 3

Accelerating effect on the growth of lactic acid bacilli in extract solutions (lactic acid bacilli were cultivated for 48 hours and the percentage content of lactic acid was determined).

Concentration of chlorellae (%) (1)	0	0.01	0.1	1.0
Extract solution.				
Extract solution obtained from the product (2) of autolysis	0.80	1.31	1.77	2.25
Extract solution obtained by using hydrochloric acid (3)	0.80	0.94	1.28	1.68

Note: (1) 8% of skim milk and 3% of dextrose was mixed with dried chlorellae (the percentages being by weight and based on the dried chlorellae).

(2) The extract solution was obtained by treating the chlorellae algae with toluene at 37°C for 24 hours with shaking.

(3) The extract solution was obtained by treating the chlorellae algae with 1% hydrochloric acid at 37°C for 24 hours.

From the test results in Table 3, it is apparent that the extract solution obtained from the autolyzed product has about 10 times the accelerating effect of the extract solution obtained by using hydrochloric acid.

The changes in the numbers of lactic acid bacilli during cultivation of lactic acid bacilli are shown in Table 4.

TABLE 4

Changes in the numbers of lactic acid bacilli during cultivation per 1 ml of medium

Culture time in hours / Medium	24	48	72	96	120	144
(1) Medium containing the extract solution obtained from the autolysis product	7.5×10^9	9.1×10^9	7.0×10^9	4.2×10^9	2.9×10^9	1.6×10^9
(2) Medium containing the solution obtained by extraction with hydrochloric acid	1.5×10^9	6.5×10^9	5.8×10^9	4.0×10^9	2.5×10^9	1.4×10^9
(3) Medium	5.8×10^8	2.2×10^9	2.7×10^9	2.8×10^9	1.6×10^9	1.1×10^9

Note: (1) The medium contains 15% of skim milk, 3% of dextrose and 0.5% of the extracted solution by weight based on the dried chlorellae.

(2) The medium contains 15% of skim milk, 3% of dextrose and 0.5% of the extracted solution by weight based on the dried chlorellae.

(3) The medium contains skim milk and dextrose in the same amount per ml of medium as in the mediums (1) or (2) but does not contain any extract solution.

As indicated in Table 4, the medium (2) increases the numbers of the living lactic acid bacilli, but with the lapse of time, they decrease rapidly, so that in the case of manufacturing beverages containing them, or in the case of preserving them, the living bacilli decrease to a fraction of their maximum numbers. However, when the extract solution obtained from the autolysis product is mixed with the nutrient medium, the decrease in the number living bacilli is slow, and stable beverages containing a great number of the bacilli can be manufactured.

As described above, a lactic acid bacilli-containing beverage manufactured according to this invention (in which the nutrient medium for cultivating the bacilli, such as Lactobacillus acidophilus, contains the extract solution from the autolysis of fresh chlorellae, animal milk, sweetening materials and essences) is quite effective as a prophylactic, especially for the treatment and prevention of alimentary canal and digestive disorders. Also it is effective for keeping health by promoting the production of lactic acid for preventing the growth of harmful micro-organisms and vitamins.

The method of this invention has the following characteristics as compared with the conventional methods. The resulting beverage or composition for making a beverage contains a great number of the living bacilli which can be maintained living for a long time. Also, it is possible to add amino acids and vitamins to the resulting beverage or composition. Therefore chlorellae can be more widely used the nutritive value of the beverage becomes high and the beverage it becomes tasty and flavourful. The beverage or composition produced from autolyzed chlorellae is ten times as effective (nutritiously and prophylactically) as a similar beverage composition produced from chlorellae using acid extraction and can be manufactured in a shorter time.

The invention is illustrated by the following examples which are in no way meant to be restrictive of the scope of the invention.

Example 1

40 grams (10 grams as the dry matter, N=100) of fresh chlorellae were concentrated by centrifugal precipitation. The chlorellae were washed with water, were suspended in sufficient phosphate buffer having a pH of 7.0 to make the total volume up to 100 ml, and 10 ml of ethyl acetate were added. Then the resulting mixture was autolyzed by shaking for 24 hours at 37°C. The autolyzed chlorellae were ground by using a colloid mill and adding water, and then the water layer was separated by centrifugal separation to give an aqueous extract solution. Non-autolyzed algae were washed with an aqueous acid solution and an alkaline solution, and the resulting washed liquor was added to the aqueous extract solution. To this mixed solution was added water to make it up to 200 ml (N=63), then 150 grams of skimed milk powder and 30 grams of dextrose, and thus a nutrient medium was prepared by diluting the resultant mixture with water to one litre. After being heat-sterilized, the nutrient medium was cooled and inoculated with Lactobacillus acidophilus and cultivated for 72 hours at 37°C. 800 ml of the cultured broth was blended with 200 ml of syrup having the following composition and the mixture was subjected to the homogenization treatment to form a novel composition for beverages.

Cane sugar	250 grams
Sodium cyclamate	4 grams
Spices	2 milligrams
Water	200 millilitres

The novel composition thus obtained has 2.1% acidity and contains 7.3 × 10^9 bacilli. The composition has a good flavour and the number of the living lactic acid bacilli does not decrease even if it is stored for 5 days at room temperature. This composition is diluted with twice its volume of sterilized water when the lactic acid bacilli-containing beverage is manufactured.

Example 2

40 grams (10 grams as dry matter, N=100) of living chlorellae (heterotropically grown obtained in the latter period of logarithmic growth) were centrifugally concentrated and washed with water to make a paste. Then, the paste was blended with 10 ml of toluene and autolyzed for 24 hours at 37°C. Then, the resulting autolyzed substance was ground in a colloid mill, 100 ml of water added to extract the water-soluble substances and the water layer was removed by centrifugal separation and non-autolysed algae were washed with water. Then the washing water was added to the water layer to make it up to 200 ml (N=92).

Next, 150 grams of skimmed milk powder and 30 grams of dextrose were added to 10 ml of the above extract solution i.e. the water layer and the resultant mixture was diluted with water to one litre to form a nutrient medium. This medium was heat sterilized and then cooled. After being cooled, the medium was inoculated with Lactobacillus acidophilus and cultured for 72 hours at 37°C. The resultant cultured broth was blended with the same syrup as described in Example 1. A novel composition was prepared by subjecting the mixture of the cultured broth and the syrup to homogenization treatment. The novel composition thus obtained had 2.2% acidity and contained 6.5 × 10^9 bacilli.

WHAT WE CLAIM IS:—

1. A method for manufacturing a beverage or a composition for making a beverage con-

taining living lactic acid bacilli, which method comprises autolysing fresh chlorellae or scenedesmus to produce an autolysed product, treating the autolysed product with water to extract the water soluble components thus forming an aqueous extract solution and mixing the aqueous extract solution with dextrose or sugar and animal milk to produce a nutrient medium, inoculating and cultivating lactic acid bacilli on the nutrient medium to produce a cultured broth and then adding sweetening materials and essences as desired to the cultured broth.

2. A method as claimed in claim 1 wherein the autolysation is carried out at a temperature of approximately 37°C.

3. A method as claimed in either preceding claim wherein the lactic acid bacillus is Lactobacillus acidophilus.

4. A method as claimed in claim 1 and substantially as hereinbefore described in either Example 1 or Example 2.

5. A beverage or a composition for making a beverage containing living lactic acid bacilli when manufactured by a method claimed in any preceding claim.

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